Coordination to Support Fisheries Management in the Western and Central Mediterranean. CopeMed Phase II

TRAINING COURSE ON ICHTHYOPLANKTON

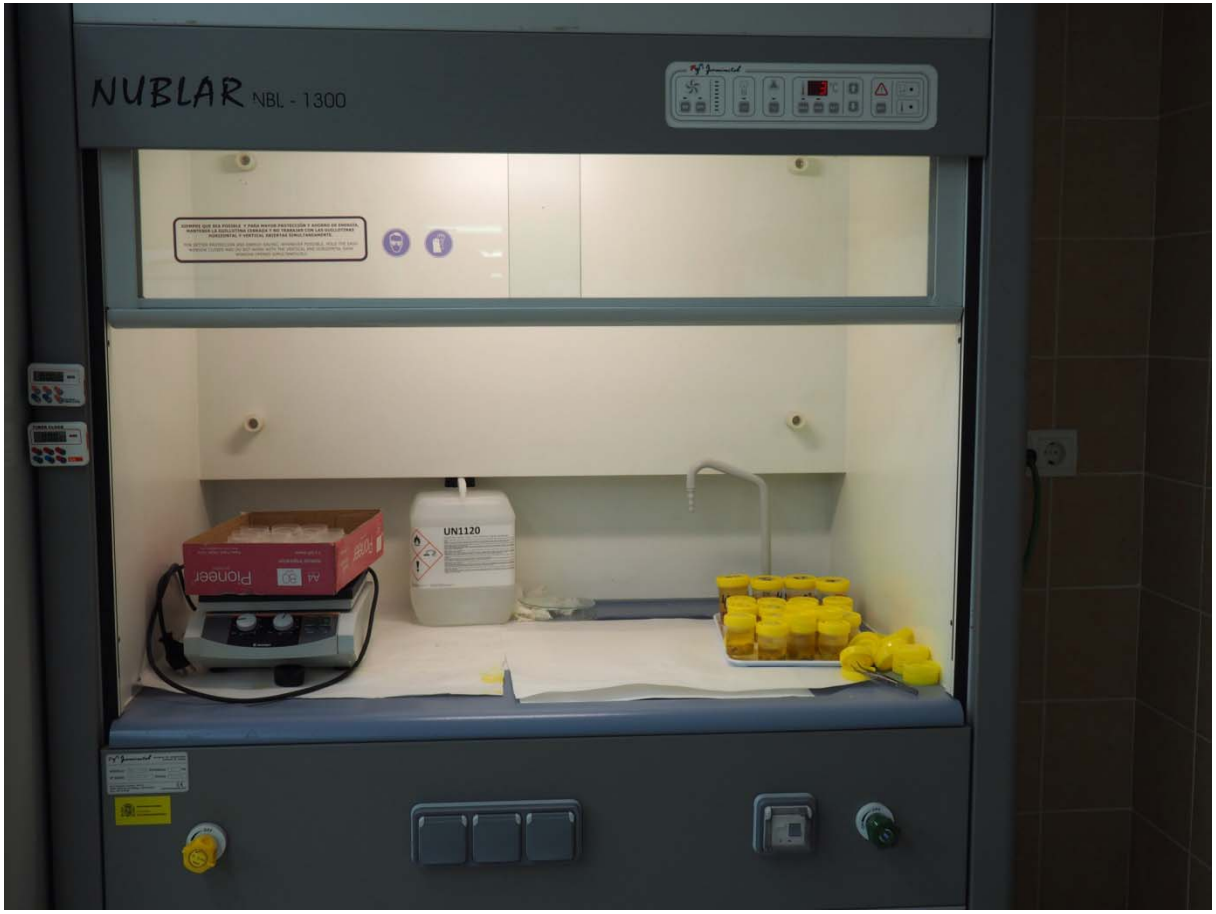
Laboratory equipment, sample preparation, ichthyoplankton sorting, preserving and storing fish eggs and larvae

by

J.M. Rodríguez and A. García

Fuengirola (Málaga), Spain
22-26 February 2016
Spanish Institute of Oceanography (IEO)

The first step in handling ichthyoplankton samples is to eliminate the preserving liquid, in most cases formalin. For this we need a **fume hood**



This allows us avoid to respire the formalin steam that is highly carcinogenic

We follow:

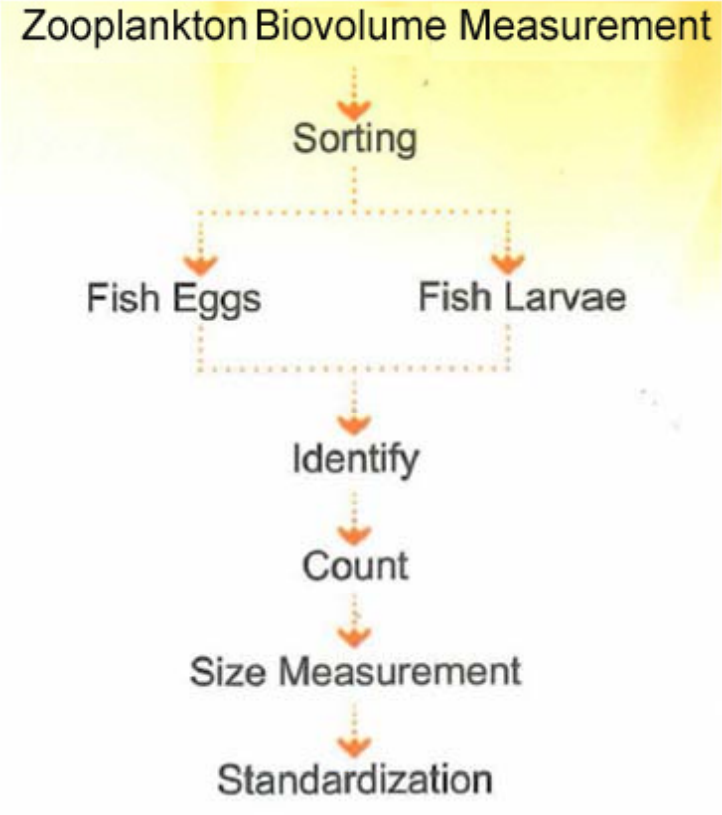
- Draining samples through a net (mesh size the same as the plankton net)
- Returning the preserving liquid to the original sampling jar

The above two steps are done inside the **fume hood**

- Washing the dry plankton sample with fresh water
- Pouring the free formalin plankton sample in a container with fresh water
- Stirring the solution softly with a glass rod
- Pouring a small quantity of the plankton sample into a petri-dish
- Sorting fish eggs and larvae from the petri-dish under a dissecting microscope at a magnification of about 10x
- Placing eggs and larvae in separated and labelled petri-dishes
- Precaution must be taken to prevent fish egg and larva damage when handling them
- Counting the number of fish eggs and larvae
- Recording the total number of fish eggs and larvae removed from the sample
- Storing fish eggs and larvae in separate labelled vials with 70% ethanol* or a solution of 5% formalin and fresh water
- The label of each vial, must include information on sampling date, station number, sampling site, sampling method (oblique , horizontal or vertical tow), and the plankton net used

*Eggs must be not preserved with ethanol because it dehydrates and wrinkles eggs, becoming not identifiable

Laboratory procedure



Zooplankton biovolume measurement

(the biovolume is an estimate of the zooplankton biomass)

- Remove non-plankton organisms, such as adult / juvenile fish and large plankton organisms (individual volume > 5 cm^3) such as jelly fish and tunicates
- Determine the total volume
- Remove the preserving liquid by filtering through a mesh of the same size that that of the plankton nets
- Determine the volume of the removed preserving liquid
- Total volume – preserving liquid = biovolume of zooplankton
- Record the biovolume
- Return the sample to the original preserving liquid

The standardization

Fish egg and larva counts can be standardised either, to number of individuals per unit of volume (m^3, 10 m^3, 100 m^3 or 1000 m^3) or to number of individuals per surface unit (generally 10 m^2). The formula for calculating the number of individuals per surface unit is:

$$no.\,of\ egg/larvae\ x\ 10\ square\ meters = \frac{n.p}{v}.10$$

Where ***n*** is the eggs or larval counts, ***p*** is the depth reached by he net during the sampling and ***v*** is the volume of water filtered by the net

Size measurement

Image capture

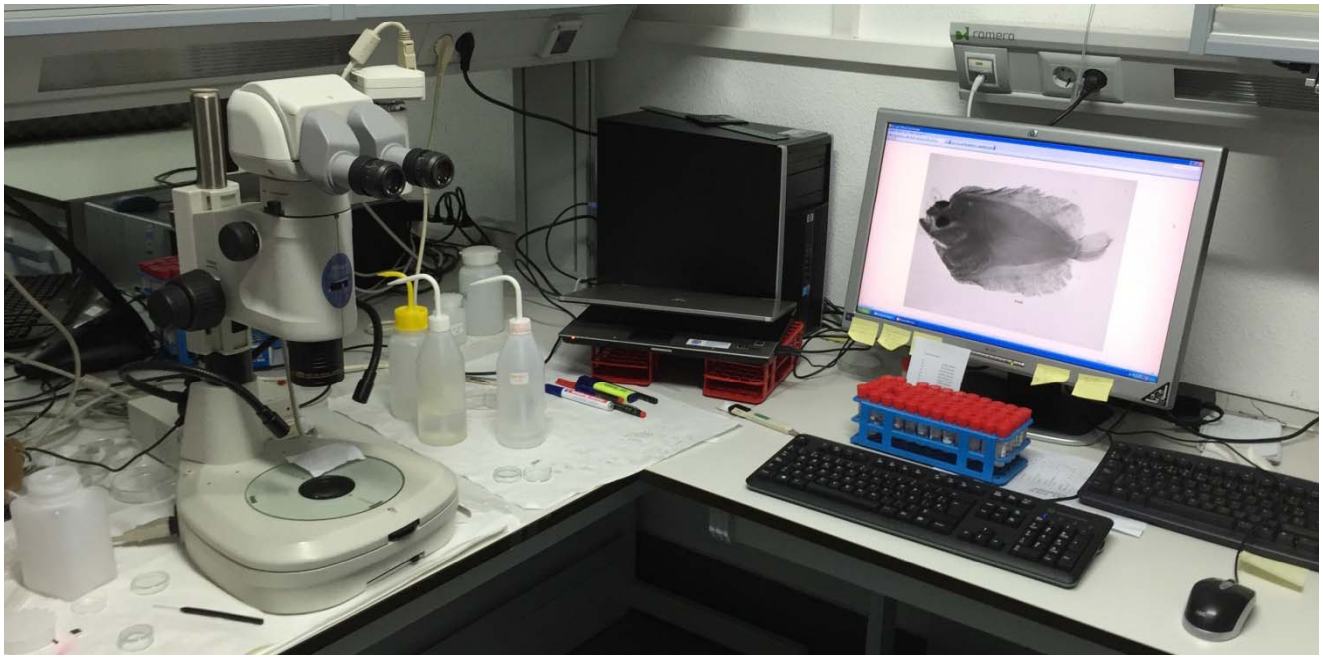
↓

Calibration of microscope magnification

↓

Image measurement

To capture an image we need a digital camera connected to a trinocular stereoscopic microscope and to a computer



The **calibration factor** tells us how many pixels correspond to a millimetre for each microscope magnification

To calibrate microscope magnification we use use graph paper

Calibration Factor

5x	66,40	pix/mm
10x	102,20	pix/mm
15x	153,80	pix/mm
20x	206,60	pix/mm
25x	257,75	pix/mm
30x	316,33	pix/mm
35x	359,67	pix/mm
40x	414,50	pix/mm

For egg and larval fish measurements we use of the free software **IMAGEJ 1.45s** (available at http://imagej.nih.gov/ij/)

Now we are going to show you how to measure a fish larva with the **IMAGEJ 1.45s**